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AWARD NUMBER: W81XWH-06-01-0018

TITLE: Is Hormonal Induction of Prostate Carcinogenesis Due to Declining Androgens in Late Life and/or Increased Estrogen in Early Life

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REPORT DATE: January 2009

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 January 2009	Final	1 Jan 2007 – 31 Dec 2008

4. TITLE AND SUBTITLE

Is Hormonal Induction of Prostate Carcinogenesis Due to Declining Androgens in Late Life and/or Increased Estrogen in Early Life

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-01-0018

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Monash University
Clayton, Victoria, Australia, 3168

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The aim of this study was to identify if exposure to estrogen during the neonatal period increases the sensitivity of the prostate to hormonal induction of malignant and pre-malignant lesions in the adult. Co-administration of high concentrations of testosterone and estradiol can induce carcinogenesis in the prostate of mice including pathologies ranging from hyperplasia to dysplasia and carcinoma in situ. When administered to estrogen-deficient ArKO and wild-type mice (WT) mice showed an increased incidence of dysplastic pathologies compared to estrogen free ArKO mice suggesting that exposure to increased estrogen increases the risk of developing prostate cancer. Subsequently ArKO and WT mice were exposed neonatally to the synthetic estrogen DES, then in adulthood exposed to combined T+E treatment to induce carcinogenesis. T+E treatment induced dysplastic lesions in both DES and non-DES treated ArKO and WT mice however it was not possible to conclusively determine if neonatal estrogen exposure altered the susceptibility of the mouse prostate to carcinogenesis. The data arising from this study have demonstrated that increased estrogen does increase the susceptibility of the prostate to hormonal carcinogenesis however it remains to be determined if this is the result of altered estrogen exposure in neonatal life or adulthood.

15. SUBJECT TERMS
prostate, neonatal, adulthood, androgens, estrogens, hormonal balance

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU		19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION

The causes of prostate cancer are unclear and it is not understood what factors may predispose a man to development of prostate cancer, although fluctuation in steroid hormones levels has been suggested (1). The ultimate aim of this study was to determine if exposure to elevated estrogen levels results in altered susceptibility to the development of prostate cancer, with the ultimate hypothesis being that exposure to estrogen during neonatal life increases the susceptibility to hormonal induction of prostate cancer in adult life. If this can be proven it will demonstrate that the risk of developing prostate cancer in adulthood can be adversely influenced by exposure to estrogen during periods of prostate development far removed from adulthood.

BODY

Task 1: Establish aromatase knockout heterozygote breeding pairs for specific production of experimental mice (Months 1-3).

Establishment of the ArKO breeding pairs was accomplished by the end of the first 3 months of this project. Due to space constraints, this was initially begun as 6 pairs and increased over subsequent months to a total of 10. As per institutional protocols for the maintenance of breeding pairs animals breeding pairs were turned over after production of 6 litters or after not achieving any pregnancy for 6 weeks.

Task 2: Induction of prostate malignancy by combined T+E treatment of adult ArKO mice (Months 3-22)

This study addressed the hypothesis that "**Preexisting prostate hyperplasia and high androgens and prolactin increases the susceptibility to hormonal induction of prostate cancer" which was generated by a previous pilot study.**

To induce carcinogenesis mice were treated with silastic implants of testosterone and estradiol, a protocol that has been described to induce carcinogenesis in rats and canines and transformation of human prostate cells (2-5). Normally ArKO and wild-type (WT) mice show significant different serum androgen profiles (Figure 1) however administration of T+E implants in both ArKO and WT mice displaying similar androgen profiles. Initially levels of both serum testosterone and DHT were highly elevated but declined over the course of treatment. Thus both ArKO and were exposed to similar hormonal influences.

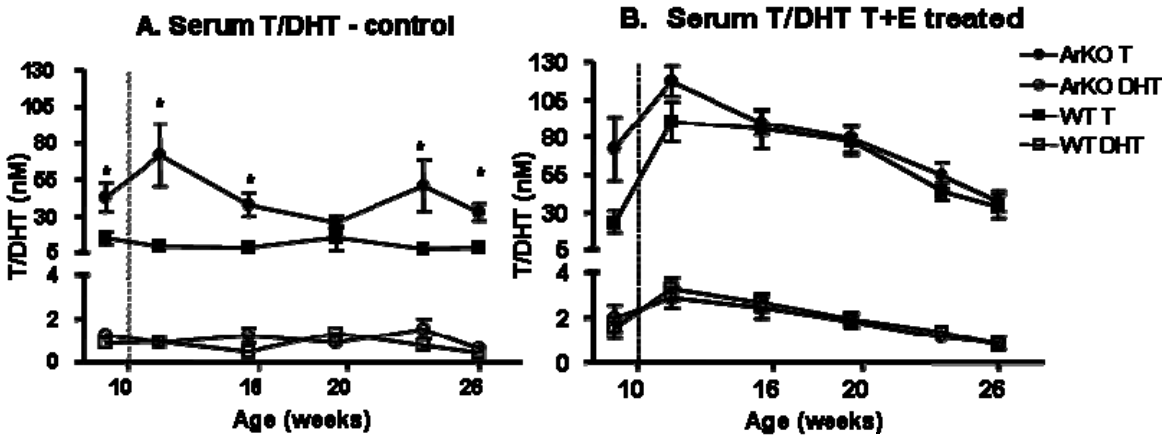

Figure 1. Serum androgen concentration following T+E treatment. A Serum T, but not DHT is significantly elevated in ArKO mice compared to WT. B. Serum T and DHT levels for both ArKO and WT mice following T+E treatment are not significantly different. Dashed line indicates time of treatment with T+E or placebo. * $p>0.05$, $n>10$

Analysis of tissue demonstrated significant weight increases in ventral, and lateral prostate lobes (VP, LP respectively) of WT mice although no significant changes were detected in dorsal and anterior prostate lobes (DP, AP). No significant change was observed in any lobe of the ArKO prostate following treatment (Figure 2). This was supported by volumetric analysis which showed significant increases in the volume of the epithelial cell compartment of WT VP and LP with no significant effect on other tissue compartments tissue or in DP or AP. Corresponding to the organ weights ArKO prostate lobes showed no effect on tissue compartmental volumes.

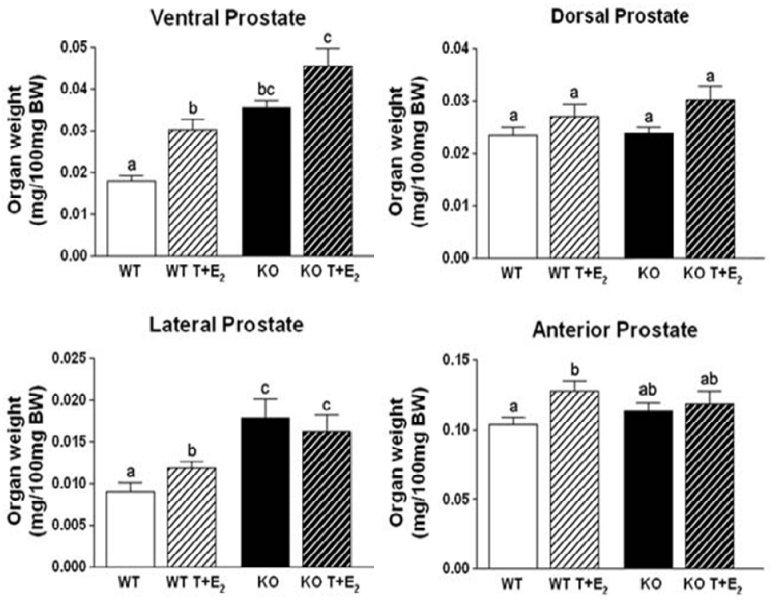

Figure 2: Effect of T+E treatment on prostate weight
Treatment of adult mice with T+E2 resulted in significant increases in WT ventral, lateral and dorsal prostate weight but had no effect on ArKO organ weight. Data expressed as mean ± SEM ; same superscripts indicate groups that are not significantly different, $p<0.05$, $n \geq 16$.

Morphological and immunohistological analysis of tissues clearly identified the appearance of epithelial hyperplasia in WT tissues and pre-malignant lesions consistent with the development of prostatic intraepithelial neoplasia and dysplasia within T+E treated tissues of both ArKO and WT animals. Immunohistological studies identified apparent increase in proliferative activity within these lesions as well as up-regulation of androgen receptor, estrogen receptor α and β. It was also apparent that some lesions demonstrated the loss of the tumor suppressor E-cadherin (Appendix 1a).

It was initially proposed that stereological analysis would be conducted to determine alteration in immunohistological markers within the prostate, however the random distribution of prostatic lesions following T+E treatment presents major problems in defining parameters and boundaries for analysis. Therefore although extensive immunohistochemistry has been conducted stereological analysis has not been conducted (Appendix 1b).

The incidence of these premalignant lesions was assessed throughout each prostate lobe with highest incidence lesions observed in the LP followed by VP, DP and AP (Table 1). However the incidence of these pathologies was reduced by up to 50% in ArKO VP and LP.

	VP		LP		DP		AP	
Pathology	WT	ArKO	WT	ArKO	WT	ArKO	WT	ArKO
Hyperplasia	100	100	100	100	100	100	67	44
Atypical hyperplasia	83	38*	100	60*	55	30	17	17
PIN/CIS	50	25*	90	50*	45	20	17	17

Table 1. Incidence of hormone induced prostate pathologies is reduced in ArKO mice, * $p < 0.05$, $n \geq 15$.

These results suggest that the ArKO mouse prostate is not predisposed to the hormonal induction of malignancy compared to WT mice which appear more susceptible to hormonal induction of carcinogenesis.

Outcome:

Although both ArKO and WT mice displayed evidence of hormonal induction of carcinogenesis, it is obvious that in the absence of endogenous estrogen production the ArKO mouse shows a reduced susceptibility to prostatic carcinogenesis arising for altered adult androgen and estrogen levels. Thus the initial hypothesis that suggested that the presence of preexisting pathology would increase the risk of carcinogenesis has been disproved.

Significance

These data demonstrate that the presence of estrogen in WT mice results in significantly increased incidence of premalignant lesions within the prostate compared to animals that do not have estrogen. Thus it is apparent that exposure to estrogen has the potential to predispose the prostate to carcinogenesis.

However it remains to be determined if the apparent resistance of ArKO mice to hormonal carcinogenesis results from the neonatal absence of estrogen/ elevation of androgen, or may be the direct result of the absence of aromatase activity in adult tissues. Identifying the cause of this response will provide new insight into the role of aromatase in prostate development and disease

Task 3: Adult exposure to high does of testosterone and estrogen, following neonatal estrogenisation (Months 8-36).

Exposure to estrogen during early life ahs been reported to have significant effects on prostate development ranging from hypertrophy, hyperplasia and dysplasia to inflammation. Given the outcome of the previous task the hypothesis that "**Exposure to estrogen during neonatal life increases the susceptibility to hormonal induction of prostate cancer in adult life"** has been proposed.

Therefore the aim of this study was to treat neonatal ArKO and WT mice with the synthetic estrogen diethylstilbestrol (DES). Animal were to be subsequently allowed to age to 90 or 180 days before receiving the same T+E treatment as applied previously. Animals were to receive the T+E implants for a further 4 months before tissue was to be collected for analysis.

Although some animals did begin treatment prior to 2008, major renovations of animal holding facilities undertaken during 2007 significantly reduced animal holding capacity and breeding resulting in a reduction in breeding pairs to 5. This obviously affected animal numbers available in 2008 further delaying breeding and treatment of experimental animals so that full cohorts of animals could not be achieved or treated within the time frame of this grant. To achieve useable numbers of animals (10 per group) it was decided to limit the study to animals aged to 90 days of age following neonatal DES treatment before administration of T+E treatment and limit the study to treatments with T+E or placebo in and ArKO and WT animals. Of those animals treated with implants a number of animals had to be humanely killed due to injuries suffered from fighting between littermates. This combined with a number of implants that could not be recovered further reduced the numbers of animals in T+E treated groups to 3-4 that could be analyzed.

Serum testosterone

Following neonatal DES administration and prior to T+E treatment serum testosterone levels in both ArKO and WT mice were not statistically different ($p>0.05$) from those seen in respective

placebo treated mice Table 2. Compared to T levels prior to T+E treatment, serum T levels at the conclusion of T+E treatment appeared to be elevated compared however due to the limited number of animals completing the full DES + T+E treatment and variability in hormone readings no statistically significant differences could be identified. Further serum T levels in T+E treated mice did not appear to be affected by prior treatment with DES when compared to animals that received T+E treatment without prior DES exposure. These data suggest that both DES non DES treated animals were exposed to comparable hormone levels during the 16 week T+E treatment thus the influence of neonatal DES on susceptibility to hormonal carcinogenesis should be able to be determined.

			+16 weeks treatment	
		90 days	Placebo	T+E
ArKO	DES	41.49 ± 39.91	30.63 + 33.67	51.18 ± 31.67
	placebo	23.8 ± 31.83	30.4 ± 30.21	42.18 ± 28.32
WT	DES	10.6 ± 15.43	9.5 ± 10.51	24.83 ± 40.83
	placebo	11.57 ± 15.49	25.2 ± 29.24	43.23 ± 38.39

Table Serum Testosterone levels in ArKO and WT mice following neonatal DES exposure and T+E2 hormonal treatment in adulthood

Organ weight.

As previously reported T+E treatment of ArKO mice did not significantly alter weights of any prostate lobe (VP, LP, AP, DP) (Appendix 1c). In animals exposed to neonatal DES alone weights of VP and LP were significantly ($P<0.05$) reduced. In VP and LP of neonatally DES treated animals treated with combined T+E, VP and LP weights were increased compared to DES alone and were similar to the weights observed in untreated controls. Weights of ArKO AP and DP were not altered by any treatment. In WT mice (Appendix 1d), T+E treatment significantly ($P<0.05$) increased weights of VP, AP and DP compared to untreated controls, and neonatal DES resulted in significantly reduced VP and LP weights. In mice exposed to both neonatal DES and T+E treatment significant increases in VP, LP and AP weights were observed compared to DES alone, however none of these was significantly different to weights observed in untreated controls. Together these data suggest that mouse VP and LP show increased sensitivity to the effect of neonatal DES, at least in terms of prostate growth, than AP or DP. Furthermore these data indicate that neonatal DES treatment does not prevent prostate growth responses to the growth promoting effects of combined T+E treatment.

Identification and incidence of prostatic carcinogenesis

As previously reported (6) induction of carcinogenesis using combined T+E treatment produces a continuum of pathologies, however it is the presence of dysplastic lesions, including high grade PIN and carcinoma in situ that clearly identify the success of T+E treatment in inducing the onset of carcinogenesis. As demonstrated in Task 2, these dysplastic lesions can be identified via the presence of altered nuclear and cellular morphology and altered expression of immunohistochemical markers PCNA, AR, ERα, ERβ and E-cadherin. Using these markers it was possible to identify evidence of dysplastic lesions within the prostates of T+E treated animals. Of those animals that received neonatal DES and subsequent placebo treatment, prostates were characterized by the presence of epithelial infolding typical of hyperplasia observed in untreated ArKO mice and the presence of inflammatory cells (Appendix 1e). The presence of inflammatory cells was also observed in animals exposed to neonatal DES followed by T+E treatment (Appendix 1e). Interestingly one of the mice that received DES alone also showed evidence of dysplastic lesions within the VP and AP. The ability of neonatal estrogen alone to induce dysplasia in the prostate has been previously reported (7-10) and clearly demonstrates that the neonatal DES treatment administered in this study does disrupt prostate cellular growth regulation.

The focal nature of the dysplastic lesions observed, precluded accurate quantification of changing levels of expression of PCNA, AR, ERα and ERβ, however in all identified lesions expression of these markers appeared to be elevated (Appendix 1f). Although E-cadherin was identifiable in some lesions it was absent from others, even within the same tissues. As E-cadherin has been implicated as a tumor suppressor (11, 12) the different patterns of E-cadherin immunoreactivity may be indicative of different degrees of progression between lesions, however this was not examined.

Using the morphological and immunohistochemical criteria listed above the incidence of dysplastic lesions in the tissues was determined (Table 3) to determine if neonatal DES altered susceptibility to hormonal carcinogenesis. In the ArKO mouse neonatal DES appeared to increase the susceptibility of the VP, LP and AP to hormonal carcinogenesis, compared to animal that did not receive DES, however the low numbers of samples available for analysis means that these results are no conclusive. In WT mice neonatal DES appeared to have no effect on carcinogenesis in VP or LP, but appeared to increase susceptibility of DP and AP however again the number of samples available means the results are inconclusive.

	VP		LP		DP		AP	
Treatment	WT	ArKO	WT	ArKO	WT	ArKO	WT	ArKO
Placebo	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0/7
DES+ placebo	0/7	1/7	0/7	0/7	0/7	0/7	0/7	1/7
Placebo/ +TE2	2/4	2/4	1/4	2/4	0/4	0/3	1/4	1/4
DES + TE2	1/4	2/3	1/4	2/3	1/4	0/3	2/4	1/3

Table 3. Incidence of hormone induced dysplastic lesions in ArKO and WT mouse prostates in the presence or absence of neonatal DES treatment.

Outcome:

Overall this study provides some evidence that exposure to estrogen may increase the susceptibility of the prostate to the onset of hormonally-induced carcinogenesis, however, due to the limitations arising from limited animal availability for use in Task 3 it was not possible to confirm that this was due to increased neonatal estrogen levels or increased estrogen synthesis in adulthood. Further studies to increase sample numbers may clarify this situation, however during the course of this project several studies have been published by other researchers that do provide strongly implicate neonatal estrogen levels with altered the sensitivity of the prostate to carcinogenesis resulting from altered androgen and estrogen levels in adulthood (8, 13-15), although exactly how this occurs and what regulatory mechanisms are involved remains unclear. In this case this question is being pursued via another in vivo mouse model of altered estrogen synthesis that results in over-expression of aromatase (16), and thus endogenous estrogen levels. Further given the report of ERα involvement in mediating hormonal carcinogenesis that arose from this project (6) and several other publications (17-20), a number of studies investigating the contributions of estrogen receptors α and β (ERα, ERβ respectively) in prostate carcinogenesis and disease are also being planned or are underway.

Significance:

It is clear that increased estrogen exposure during the lifetime of an individual may increase the risk of developing prostate cancer as hormone levels change with age. However it remains to be determined if this increased susceptibility results from altered exposure to estrogen during specific periods of life ie neonatal or adult life, or altered hormonal activity in adult life, occurring as a result of changing ratios of hormone levels.

However there is increasing evidence linking estrogenic compounds and synthetic compounds that alter the balance of androgens and estrogens with prostatic carcinogenesis which has led to ongoing studies within this lab targeting the role of estrogen receptors, particularly ERβ in regulation of prostate growth and disease and its potential as an anti-carcinogenic target in the prostate as well as the effect of endocrine disruptive chemicals on prostate development and health.

KEY RESEARCH ACCOMPLISHMENTS

List of key research accomplishments emanating from this research

- Immunohistological characterization of pre-malignant lesions
- Demonstration of hormonal profiles following T+E treatment of ArKO and WT mice
- Stereological quantiation of prostate pathology in T+E treated animals identifying epithelium as site of aberrant growth
- Demonstration of reduced susceptibility to hormonal carcinogenesis in absence of estrogen synthesis
- Utility of T+E treatment as a model of carcinogenesis in mice
- Report of specific involvement of ERα in prostatic hormonal carcinogenesis
- Implication of neonatal estrogen exposure with altered susceptibility to hormonally-driven carcinogenesis in adult life.

REPORTABLE OUTCOMES:

	Reportable outcomes that have resulted from this research
Manuscripts	William A. Ricke, Stephen J. McPherson, Joseph J Bianco, Gerald R. Cunha, Yuzhuo Wang, Gail P. Risbridger. Prostatic hormonal carcinogenesis is mediated by *in situ* estrogen production and estrogen receptor alpha-signaling. (2008) *FASEB J* **22,** 1512-1520.
Abstracts/Presentations	SJ McPherson, JS Pedersen, GP Risbridger. Absence of Endogenous Aromatase Activity and Estrogen Results in Reduced Susceptibility to Hormonal Induction of Prostate

	Malignancy in Adulthood. *Department of Defense, Prostate Cancer Research Program, Innovative Minds in Prostate Cancer Today (IMPaCT) meeting.*, Atlanta, USA, 5-8 September 2007. (See Appendix 2a) McPherson SJ, Bianco JJ, Wang H, Pedersen JS, Wang YZ, Cunha GR, Risbridger GP. Estrogen deficient mice are less susceptible to hormone dependent induction of prostate malignancy. *12th International Congress on Steroid Hormones and Cancer*, Athens, Greece, 2006. (See Appendix 2b)
Patents & licenses	nil
Degrees obtained	nil
Development of cell lines	nil
Tissue or serum repositories	nil
Informatics such as databases and animal models	nil
Funding applied for based on this award	nil
Employment or research opportunities applied for and/or received based on experience/training supported by this award	nil

CONCLUSION:

In summary this project has provided significant data regarding the influence of estrogen on the susceptibility to prostatic carcinogenesis. Specifically this study has shown that the presence of increased estrogen in a male can cause an increase in the incidence of prostatic lesions caused by alteration in adult hormone levels that can lead to carcinogenesis and has also contributed to the demonstration that ERα is a key factor in determining sensitivity to prostatic hormonal carcinogenesis. Currently it is unclear if this is due to the long term effect originating from

estrogen exposure during early life or is specifically due to aberration in adult hormonal balances although there is increasing data supporting the estrogen as a predisposing factor and the neonatal period as the critical time for determining prostatic susceptibility to carcinogenesis. The potential for early life events to modulate late life susceptibility to disease is of significant concern, highlighted by the increasing awareness of endocrine-disruptive activities, including both estrogenic and anti-androgenic actions, of chemicals commonly found within the environment eg bisphenol A (14, 15, 21). Determining if exposure to these natural and synthetic compounds adversely impacts on prostate health may provide insight into the causes behind prostate disease, potentially allowing for the development of new diagnostic, or even therapeutic technologies, or the identification and elimination of factors that increase an individual's susceptibility to prostate disease.

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APPENDIX 1: ADDITIONAL FIGURES.

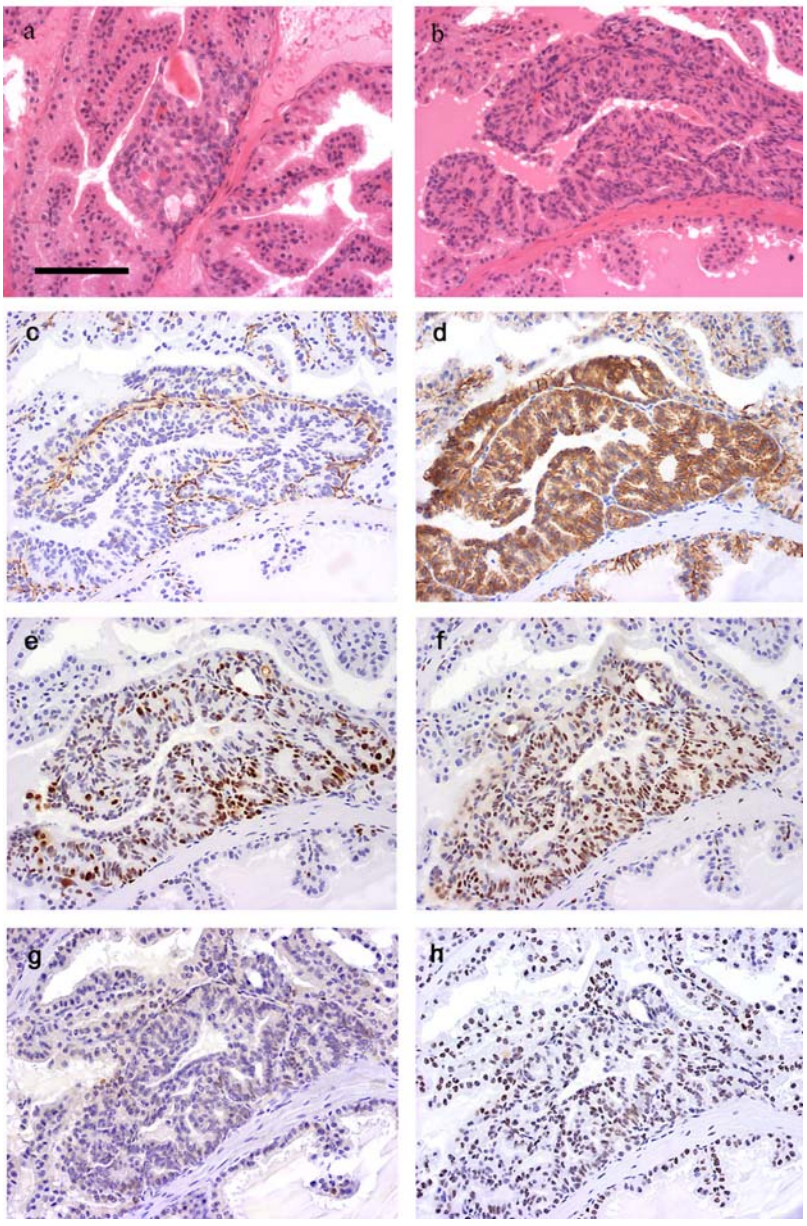

Figure 1a: Identification of dysplasia in mouse prostate lobes following T+E2 hormonally-treated mice. Focal dysplastic lesions could be identified by histological parameters in prostates from both WT and ArKO mice following T+E2 treatment. Both low (b) and high (b) grade lesions were identifiable. Immunohistochemistry was also used to identify lesions. c-h: High grade dysplastic lesion showing high molecular weight cytokeratin (CKH; c), E-cadherin (d), PCNA (e), AR (f), ERα (g), ERβ (h). Scale bar = 25μm.

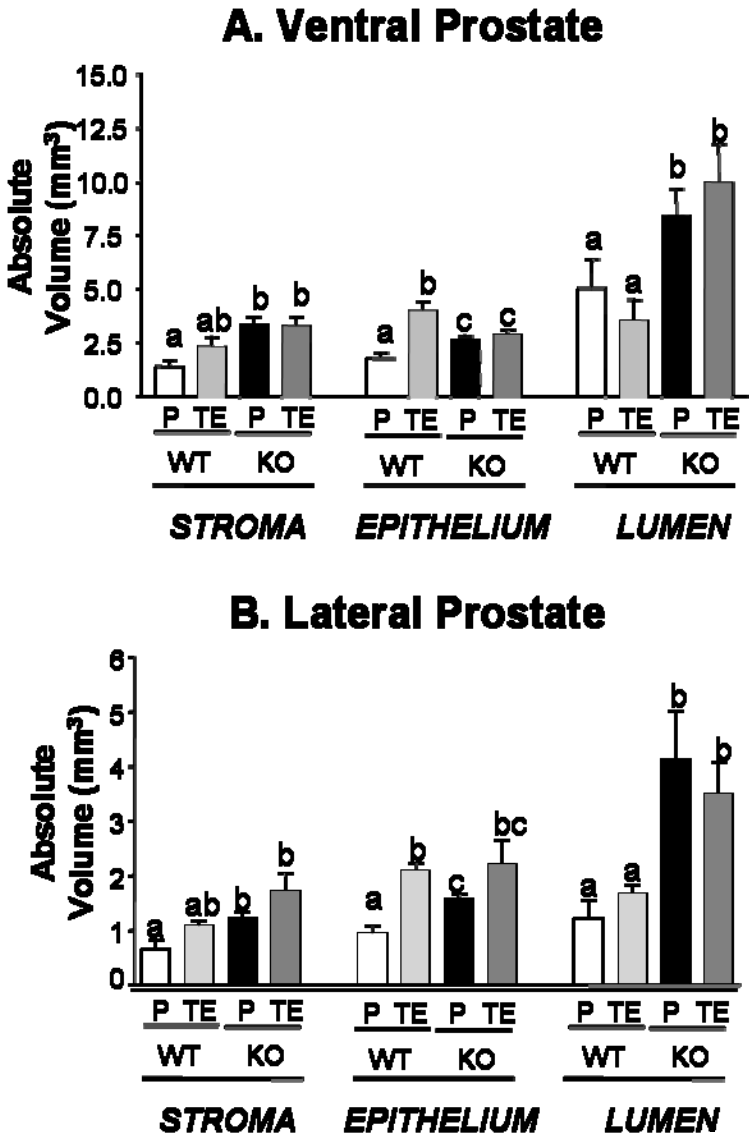

Figure 1b. Stereological analysis of prostate lobes following T+E2 treatment. Consistent with histological and weight data, stereological analysis of (A)Ventral and (B) Lateral prostate lobes demonstrated significant increases in absolute volumes of epithelial compartment of WT VP and LP ($p<0.05$). Same superscript indicates no significant difference ($p < 0.05$, $n >10$) in same tissue compartment.

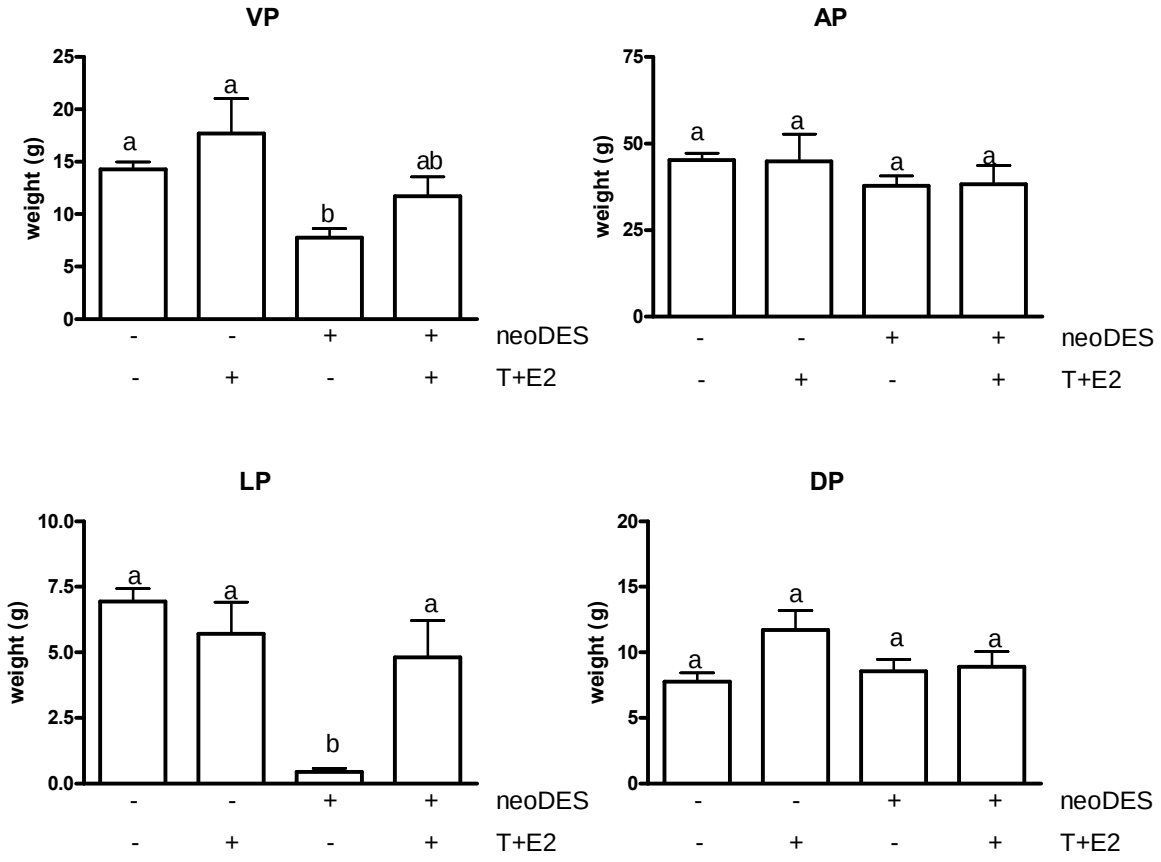

Figure 1c: Effect of neonatal DES and combined T+E treatment on ArKO mouse prostate weight. Neonatal exposure to DES resulted in significant reductions in weights of ventral (VP) and lateral prostate lobes compared to non-treated tissues. Administration of T+E alone did not significantly alter VP or LP weight however administration of this treatment to DES treated mice resulted in VP and LP weights being no different to those of non-treated controls. Weights of anterior (AP) and dorsal (DP) were not significantly altered by any treatment Data expressed as mean ± SEM ; same superscripts indicate groups that are not significantly different, $p<0.05$, $n \leq 7$.

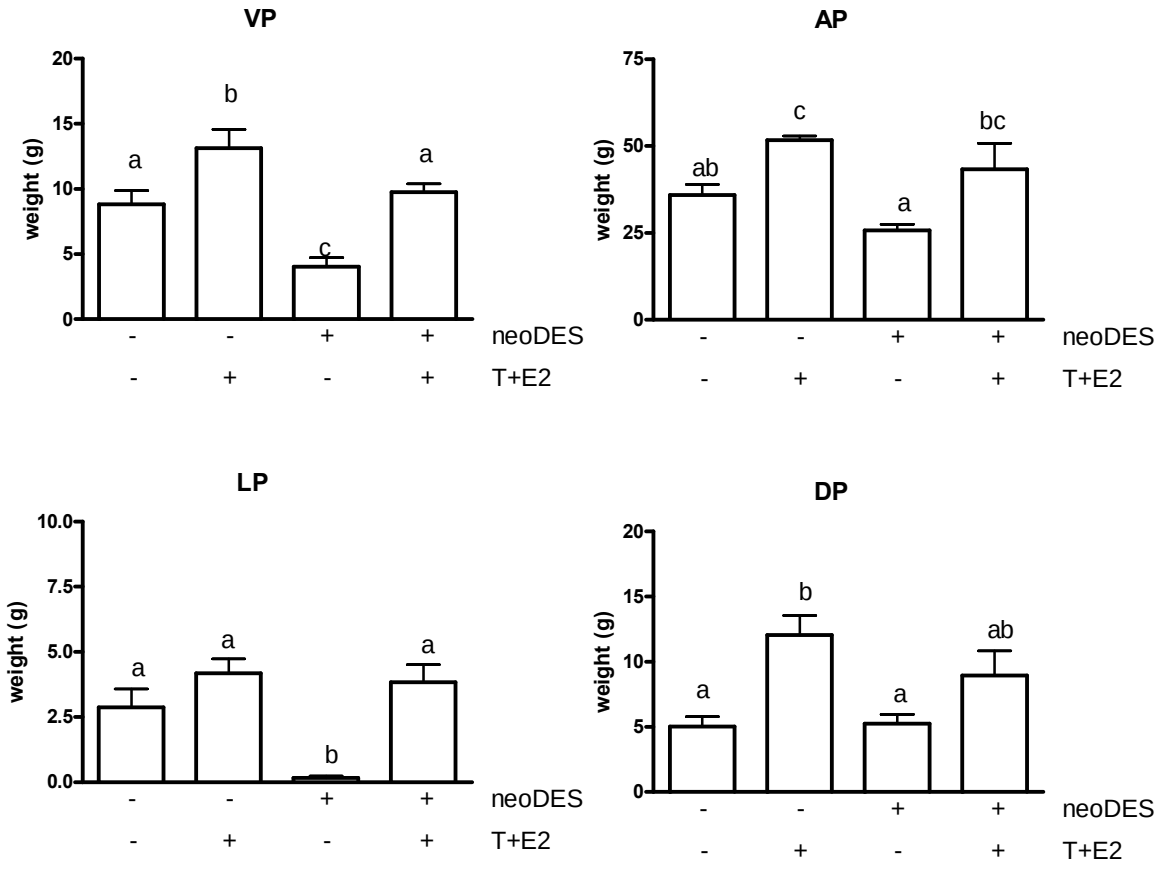

Figure 1d: Effect of neonatal DES and combined T+E treatment on wild-type mouse prostate weight. Neonatal exposure to DES resulted in significant reductions in weights of ventral (VP) and lateral (LP) prostate lobes compared to tissues that were not treated with DES. Administration of T+E alone significantly increased VP, anterior (AP), and dorsal (DP) prostate weight but not LP. Administration of T+E to DES treated mice resulted in final VP, LP, DP and AP weights being no different to non-treated controls. Data expressed as mean ± SEM ; same superscripts indicate groups that are not significantly different, $p<0.05$, $n \leq 7$.

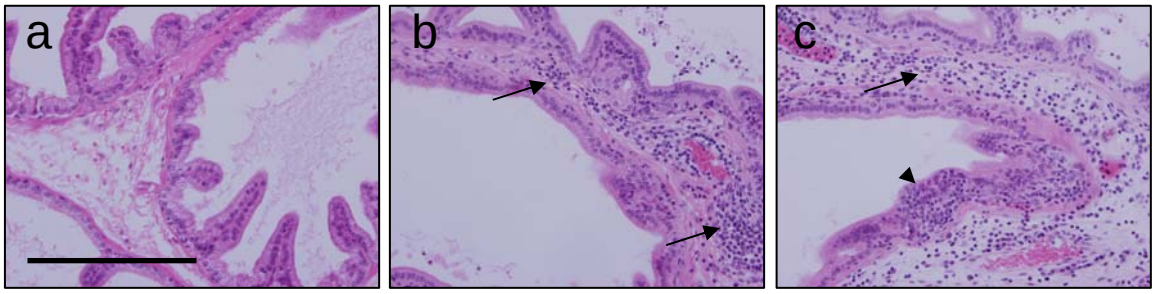

Figure 1e: Detection of inflammatory cells in ArKO mouse prostate following neonatal DES. a) Placebo treated ArKO prostate showing typical epithelial infolding b) neonatal DES treated prostate demonstrating presence of inflammatory cells (arrows). c) Inflammatory cells in close proximity to dysplastic lesion (arrowhead) of neonatal DES, T+E treated prostate (arrows). Scale bar =200μm

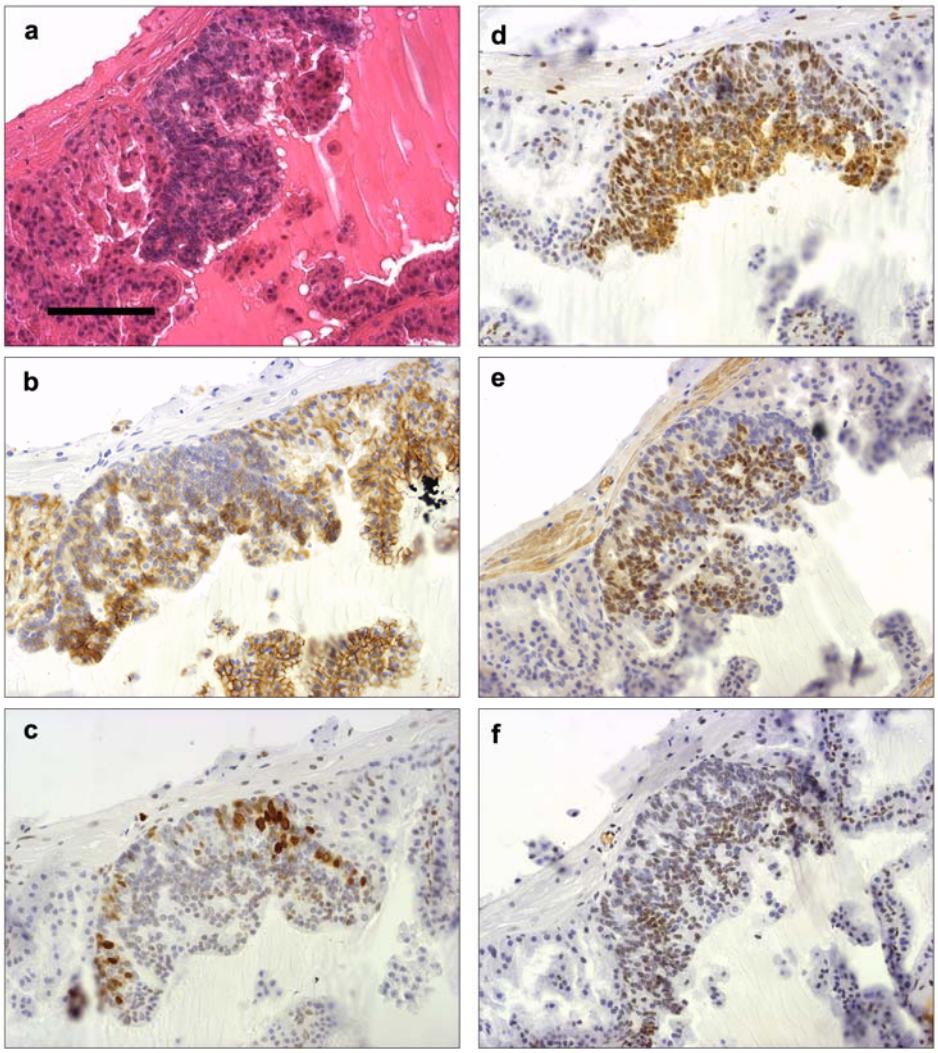

Figure 1f: Identification of dysplasia in mouse prostate lobes following T+E2 treatment after neonatal DES exposure. Focal dysplastic lesions could be identified by histological parameters in prostates from both WT and ArKO mice following T+E2 treatment. Immunohistochemistry was also used to identify lesions. (a) H&E, c-h: dysplastic lesion showing localization of E-cadherin (b), PCNA (c), AR (d), ERα (e), ERβ (f). Scale bar = 100μm.

APPENDIX 2. ABSTRACT PRESENTATIONS

1. Poster presentation given by Stephen McPherson at the inaugural *Department of Defense, Prostate Cancer Research Program, Innovative Minds in Prostate Cancer Today (IMPaCT) meeting.*, Atlanta, USA, 5-8 September 2007.

Absence of endogenous aromatase activity and estrogen results in reduced susceptibility to hormonal induction of prostate malignancy in adulthood.

Stephen J McPherson; John S Pedersen (Tissupath, Melbourne, Victoria, Australia); Gail P Risbridger

The incidence of prostate cancer in men increases with age and although dependent on the presence of androgens, commonly occurs at an age where the levels of serum testosterone are declining and the ratio of estrogens: androgens is increasing. The contradictory nature of this observation has focused attention on estrogens and particularly on how the balance between androgens and estrogens might alter the incidence of prostate cancer that develops upon aging. Evidence exists that exposure to high levels of estrogen during early neonatal life (imprinting) can have permanent and irreversible effects leading to aberrant prostate development and the development of premalignant and inflammatory pathologies in adult life. It has also been shown that altering the hormonal milieu in adult animals, using combined androgen and estrogen treatment, can induce prostatic malignancy. In both cases it is the presence of estrogen that determines the resultant pathology.

Given the involvement of estrogen in the process of prostatic carcinogenesis the purpose of this project is to determine if suppression of adult and/or neonatal estrogen levels results in a reduced sensitivity to the development of hormonally induced prostate cancer.

In the initial phase of this investigation we have administered silastic implants containing Testosterone + Estradiol ($T+E_2$) implants into estrogen-deficient aromatase knockout (ArKO) and WT mice for 4 months to determine if the absence of estrogens results in altered sensitivity to hormonally induced carcinogenesis. The results of this study have demonstrated the induction of malignant and premalignant pathologies in prostates of ArKO mice, is reduced by up to 50% compared to WT animals. Interestingly it has also shown that the most androgen sensitive prostate lobes (ventral and lateral) are demonstrating the greatest reduction in incidence of pathological lesions. Thus it is apparent that the absence of endogenous aromatase activity and thus systemic estrogen results in a reduced incidence of hormonally-induced malignant and non-malignant prostatic pathologies.

It is unclear if reduced sensitivity of the ArKO prostate to hormonal induction of carcinogenesis is due to reduced adult estrogen levels or an effect of altered neonatal imprinting occurring between the estrogen-deficient ArKO mice and WT mice. This issue is currently being addressed

in studies combining neonatal estrogenization of both ArKO and WT mice with exposure to $T+E_2$ treatment in adulthood.

IMPACT: Given the sensitivity of the neonatal prostate to abnormal hormone levels and the fact that the balance of hormones alters in aging men, this project may have significant impact on the ability to identify individuals with increased risk of developing prostate disease/cancer. Further this project will determine if susceptibility to prostate disease/cancer may be influenced by hormonal exposure during early life.

2. Poster presentation given by Gail Risbridger on behalf of Stephen McPherson at *12th International Congress on Steroid Hormones and Cancer,* Athens, Greece, 2006.

ESTROGEN DEFICIENT MICE ARE LESS SUSCEPTIBLE TO HORMONE DEPENDENT INDUCTION OF PROSTATE MALIGNANCY.

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Androgens and estrogens are critical in regulating early and late life development of the prostate gland. Yet estrogens are regarded as potentially carcinogenic and neonatal estrogenization is linked to the potential development of prostate malignancy in late life(9, 22). Estrogen deficiency occurs in aromatase knockout (ArKO) mice that spontaneously develop prostatic hypertrophy and hyperplasia, but do not develop prostatic malignancy. As ArKO mice show inherent prostate growth abnormalities and a lifelong hormone imbalance, we predicted that they would show increased susceptibility to the induction of prostate cancer in adulthood. To test this hypothesis, the rate of hormonal induction of malignancy was compared in adult ArKO and wild-type (WT) mice, using combined testosterone + estradiol (T+E) treatment for 4 months.

Following T+E treatment both ArKO and WT serum androgen levels were elevated to similar levels. T+E-treated WT prostates demonstrated increased localization of androgen receptor (AR) and significant increases in prostate lobe weights and epithelial volume. Hyperplasia and dysplasia, was identifiable in all prostate lobes in WT mice, although the rate of incidence varied between lobes; lateral - 90% incidence of dysplasia, ventral - 50%, dorsal - 45%, anterior - 17%. T+E treated ArKO tissues showed no significant changes to weight, volume or AR localization, however dysplastic lesions were detectable, but at an incidence approximately half that seen in WT (lateral 50%; ventral 23%; dorsal 20%) although anterior prostate was no different.

The results demonstrate that in the absence of endogenous estrogen, hormonal induction of premalignant dysplastic lesions of the prostate is significantly reduced. This suggests that exposure to estrogen may increase the predisposition of the prostate to malignant change resulting from hormonal imbalances in adulthood. It remains to be determined if the apparent resistance of ArKO prostate to induction of premalignant lesions is the result of hormonal imprinting during neonatal life or of altered hormonal influences in adulthood.